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Variations in Brain Volume and Growth in Young Children With Type 1 Diabetes

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PATHOPHYSIOLOGY

Early-onset type 1 diabetes may affect the developing brain during a critical window of rapid brain maturation. Structural MRI was performed on 141 children with diabetes (4–10 years of age at study entry) and 69 age-matched control subjects at two time points spaced 18 months apart. For the children with diabetes, the mean (±SD) HbA_{1c} level was 7.9 ± 0.9% (63 ± 9.8 mmol/mol) at both time points. Relative to control subjects, children with diabetes had significantly less growth of cortical gray matter volume and cortical surface area and significantly less growth of white matter volume throughout the cortex and cerebellum. For the population with diabetes, the change in the blood glucose level at the time of scan across longitudinal time points was negatively correlated with the change in gray and white matter volumes, suggesting that fluctuating glucose levels in children with diabetes may be associated with corresponding fluctuations in brain volume. In addition, measures of hyperglycemia and glycemic variation were significantly negatively correlated with the development of surface curvature. These results demonstrate that early-onset type 1 diabetes has widespread effects on the growth of gray and white matter in children whose blood glucose levels are well within the current treatment guidelines for the management of diabetes.

Type 1 diabetes, which is characterized by autoimmune-mediated destruction of the pancreatic β-cells, has been associated with subtle cognitive deficits (1–4) as well as long-term microvascular and macrovascular complications (5–7). Brain-imaging studies (6–11) have shown that type 1 diabetes is associated with reduced total and regional loss of gray matter volume (GMV) in adolescents and adults. Similarly, in white matter, type 1 diabetes has been associated with total and regional volume losses (3,5,10), differences in connectivity and microstructure (12–15), and increased likelihood of white matter hyperintensities in older adults (16).

While type 1 diabetes usually first appears in childhood or adolescence, an early age of onset, generally defined as earlier than 4–7 years of age by different authors (2,17–19), is associated with more severe cognitive symptoms than late-onset diabetes; thus, the effects of this disease may be particularly evident in brain development in very young children. In particular, young children are particularly prone to experience extreme swings of hyperglycemia and hypoglycemia; hence, the current treatment guidelines for young children allow some exposure to hyperglycemia in order to reduce the neurological risks of severe hypoglycemia (20). Previous cross-sectional studies

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(21–24) of young children with early-onset diabetes have shown differences in regional GMVs and axial diffusivity in white matter. The Diabetes Research in Children Network (DirecNet) Consortium (25) studied a large longitudinal sample of clinically treated young children with type 1 diabetes to investigate the effects of glycemic control on brain development and cognition. Using voxel-based morphometry (VBM), investigators found that children with diabetes had significantly lower growth of GMV over much of the cortical surface (25), even though the subjects with diabetes were well within the clinical guidelines for diabetes management (20).

Since many brain differences seen later in life may originate in this crucial early period of brain development (3,15,16,26), we undertook further studies of this young population. A limitation of VBM is that it cannot discriminate features such as cortical thickness (CT), curvature, or surface area (SA) (27), which may be affected differently and independently in this disease. A surface-based analysis method such as FreeSurfer (28) can measure these surface characteristics, as well as accurately estimate subcortical and white matter volumes (WMVs) in the brain. The growth of WMVs is of particular interest because animal models (29–31) suggest that diabetes may adversely affect myelination early in brain development such that widespread myelination effects may affect white matter growth. Measurements of brain volume also may be confounded in cross-sectional studies because dehydration affects total brain volume (32,33), and children with diabetes are often mildly dehydrated due to excess blood glucose. However, longitudinal data that include blood glucose measurements provide an opportunity to estimate this potential glycemia-brain volume correlation.

In the current study, we used FreeSurfer-based analyses to investigate the impact of early-onset diabetes on cortical development and regional brain growth in young children. We also sought to explore how age may modify the effects of diabetes on the developing brain and how blood glucose levels at the time of a scan may affect the measurement of brain volume in the population of individuals with diabetes.

RESEARCH DESIGN AND METHODS

Research protocols were reviewed and approved by the institutional review board of each of the five participating centers and were conducted after signed informed consent and child assent (when appropriate) were collected.

Study Subjects

Details of the study participants have been previously reported (23–25,34). In brief, children with diabetes (N = 144) and healthy control subjects without diabetes (N = 72) between 4 and 10 years of age (mean age at study entry 7.0 years) were recruited for the study, and 210 participants (children with diabetes N = 141, control subjects N = 69) were successfully imaged at both time points. By design, all participants were born at ≥34 weeks of gestation with a birth weight of ≥2,000 g and had no genetic, neurologic, or psychiatric disorders; had no intellectual disability or language or learning disability; were not enrolled in self-contained special education programs; and had no visual or auditory impairments and no contraindications for brain MRI. In addition, participants with diabetes had an onset age older than 6 months, and healthy control subjects without diabetes had a glycated hemoglobin (HbA_{1c}) level of <6.0% (42 mmol/mol) and a fasting blood glucose level of <110 mg/dL (6.1 mmol/L).

Study Procedures

At study enrollment and after 18 months, all subjects underwent brain imaging and neurocognitive testing (34). Glycemic measures were assessed every 3 months in the group with diabetes. HbA_{1c} was measured quarterly (DCA 2000), and severe hypoglycemia and ketoacidosis events were recorded. Continuous glucose monitoring (CGM) was performed to collect glycemic data for at least 72 h (and at least 24 h overnight) every 3 months for 18 months using either their own CGM devices (MiniMed Paradigm REAL-Time Revel; Medtronic, Northridge, CA, or SEVEN Plus; Dexcom, San Diego, CA) or an iPro2 device with Enlite sensor (MiniMed; Medtronic).

Imaging Data Acquisition

All imaging sites used a Siemens 3T MAGNETOM Trio, a Tim System whole-body MRI system, and a standard 12-channel head coil. An identical imaging protocol was uploaded to every scanner. Multisite calibrations were performed by scanning the same two adult human phantoms on every machine to confirm the repeatability of measurements across sites (23). Sagittal T1 images of the brain were acquired using a magnetization-prepared rapid acquisition gradient echo (MP-RAGE) pulse sequence with the following parameters: repetition time 2,300 ms, echo time 2.98 ms, inversion time 900 ms, flip angle 9°, field of view 25.6 × 24 cm, 160 slices, matrix 256 × 256, voxel size 1.0 × 1.0 × 1.0 mm, and duration 4 min 54 seconds. Participants were awake and unsedated and had previously received training designed to help them succeed with the motion restriction requirements of MRI (35). By design, two MP-RAGE acquisitions were obtained for all participants to increase the probability that at least one scan would be collected with minimal head motion. A second MRI session was performed on a separate day if the initial scan could not be successfully completed or if image quality was deemed unacceptable after the first attempt. Participants with diabetes were required to have glucose levels between 70 and 300 mg/dL within 60 min prior to all scanning sessions.

Structural Analyses

Cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite, version 5.3 (http://surfer.nmr.mgh.harvard.edu/), optimized for longitudinal processing and SA accuracy (36,37). The FreeSurfer software pipeline calculates the cortical surface and sulci of every subject using a surface registration method (38), segments the volumes of subcortical regions (28), and calculates their volumes in native space without any geometric deformations. The gray-white and pial surfaces were visually inspected, and, where needed, the appropriate manual corrections were performed as per the FreeSurfer tutorial for the longitudinal pipeline (http://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/). All segmentations were visually examined by a trained reviewer blinded to participant group, with particular attention to the consistency of segmentations across time points for each participant.

The cortical surface was registered across subjects by using a spherical atlas that uses individual cortical folding patterns to match cortical geometry across subjects (38). FreeSurfer calculates a vertex-wise GMV, SA of the gray matter-white matter boundary, mean CT, and curvature for each vertex on the surface. The mean curvature of the surface is defined as the integrated rectified curvature, such that higher values indicate deeper, more sharply defined sulci. All surface-based analyses were smoothed with a Gaussian kernel with a full width at half-maximum of 15 mm.

Glycemic Measures

Previous studies have suggested that hyperglycemia, hypoglycemia, and glycemic variation may each affect structural growth. Glycemic exposure (18moA1C) was estimated from clinical variables as the area under the curve (AUC) above 6.0% using all available HbA_{1c} history data between time points (25). Glycemic exposures from CGM variables were estimated for mean glucose (GluMean), high glucose (AUC but above 180), low glucose (area over the curve but below 70), SD, and the mean amplitude of glucose excursions (MAGEs) (39). These measures were computed from the average of all CGM quarterly measurements (usually seven per participant) during the 18-month interval. For each participant with diabetes, we calculated the difference (in milligrams per deciliter) of the blood glucose levels at the two time points (scan time1 and scan time2). The blood glucose level at the time of each scan was computed as the average of blood glucose measurements obtained immediately before and after the MRI scan. Blood glucose measurements were recorded only for the subjects with diabetes.

Statistics

Prospective growth of total cortical GMV and SA, and mean CT and curvature for the between-group analyses, were analyzed in SPSS using repeated measures while controlling for age at time1, sex, and the interval between the two time points. Within the group with diabetes, correlations of structural changes with glycemic variables (including the difference in blood glucose levels) were investigated in SPSS using repeated measures for the structure and including the glycemic variable as a covariate of interest while controlling for age at time1, sex, and the interval between scans. Statistical results are reported as $P < 0.05$ for each of these analyses to show the effects of diabetes on growth throughout the brain. Post hoc analyses with group × age interactions were investigated for between-group effects at 1 SD above and below the mean age (40).

For regional analyses of cortical surface growth, FreeSurfer calculates a local rate of change by including the interval in the calculation (41); thus, between-group analyses of structural change were covaried for age and sex. Within the group with diabetes, correlations of structural change were studied using the glycemic variables as covariates and controlling for age and sex. We report whole-brain significant results at $P < 0.05$ for a two-tailed t test corrected cluster-wise using Monte Carlo Null-Z simulation (42). Correlations with glucose variability (MAGE) were also covaried by GluMean level to discriminate the transient effects from the average glucose level observed during usual clinical visits.

RESULTS

The demographics of the groups are shown in Table 1. There was a small (~22-day) but significant difference ($P < 0.001$) in the interval between scans. Within the group with diabetes, there was no significant difference in values across time points for HbA_{1c}, MAGE, or blood glucose level.

Total Volume Growth

Subjects with diabetes showed significantly slower growth than control subjects for both total white matter ($P < 0.003$) and total gray matter ($P < 0.05$) (Table 2). Total WMVs within each group, but not GMVs, were significantly larger at time2 than time1 (each group, $P < 0.001$). There was a significant negative correlation of growth rate with age (white matter $P < 0.001$, gray matter $P < 0.01$), such

Table 1—Demographic and glycemic characteristics

	Children with type 1 diabetes	Control subjects
Number (male/female)	141 (76/65)	69 (37/32)
Age (years)		
Time1 (baseline)	7.01 (1.66)	6.96 (1.77)
Time2 (18 months)	8.48 (1.64)	8.50 (1.77)
Interval	1.47 (0.09)	1.53 (0.07)
Age at onset (years)	4.07 (1.86)	NA
Duration at time1 (years)	2.90 (1.89)	NA
18 months of exposure (18moA1C)	2.88 (1.15)	NA
18-month average MAGE (mg/dL)	159 (24)	NA
18-month mean number severe hypoglycemic episodes	0.05 (range 0–2)	NA
HbA_{1c}, %; mmol/mol		
Time1	7.9 (0.9); 63 (10)	NA
Time2	7.9 (0.9); 63 (10)	NA
Blood glucose at scan Time1; Time2	176 (54); 177 (58)	NA
MAGE (mg/dL) Time1; Time2	157 (32); 160 (30)	NA

Data are reported as the mean (SD). NA, not applicable.

Table 2—Growth differences of regional volumes and global surface properties

	Control subjects		Type 1 diabetes		Growth difference (control subjects > diabetes subjects)
	Time1	Time2	Time1	Time2	P value
Cortical surface					
Cortical volume (1,000 mm^3)	590 (42)	591 (42)	588 (45)	586 (46)	0.046
Total SA (1,000 mm^2)	178.2 (13)	179.1 (13)	177.5 (15)	177.9 (15)	0.03
Mean CT (mm)	2.89 (0.09)	2.86 (0.09)	2.90 (0.09)	2.86 (0.08)	NS
Mean curvature	0.168 (0.006)	0.169 (0.007)	0.168 (0.007)	0.169 (0.007)	NS
Brain volumes (1,000 mm^3)					
Total gray matter	759 (51)	762 (50)	756 (55)	754 (55)	0.05
Total white matter	427 (45)	442 (45)	425 (47)	437 (49)	0.003
Basal ganglia total	59.4 (4.8)	60.2 (4.8)	59.0 (4.6)	59.7 (4.6)	NS
Cerebral white matter (lh)	190 (21)	196 (21)	189 (22)	194 (22)	0.049
Cerebral white matter (rh)	190 (21)	196 (21)	189 (22)	194 (22)	0.004
Corpus callosum	2.70 (0.35)	2.80 (0.35)	2.72 (0.38)	2.80 (0.39)	0.001
Ventricular volume	11 (5.3)	11 (5.6)	11 (4.9)	11 (4.9)	NS
Cerebellar gray matter (lh)	54.0 (45)	54.8 (4.4)	53.4 (5.0)	54.0 (4.8)	NS
Cerebellar gray matter (rh)	55.3 (45)	55.9 (4.4)	54.6 (5.1)	55.1 (5.1)	NS
Cerebellar white matter (lh)	12.9 (1.4)	13.7 (1.4)	12.7 (1.6)	13.2 (1.6)	0.014
Cerebellar white matter (rh)	13.3 (1.5)	14.0 (1.5)	12.8 (1.5)	13.4 (1.6)	0.018
Brain stem (pons, midbrain)	18.4 (1.9)	19.2 (1.9)	18.3 (2.0)	18.9 (2.0)	0.003

Data are reported as the mean (SD). lh, left hemisphere; NS, not significant; rh, right hemisphere.

that the rates of growth of total GMV and total WMV were highest at younger ages (Fig. 1).

Cortical Surface Growth

Subjects with diabetes showed significantly slower growth rates than control subjects in total cortical volume ($P < 0.05$) and total SA ($P < 0.03$), but not for mean CT ($P < 0.12$) or mean curvature (Table 2). The mean curvature significantly increased from time1 to time2 for both groups (control subjects $P < 0.005$, children with diabetes $P < 0.02$); however, the change in mean curvature within the group with diabetes was significantly negatively correlated with 18-month exposure to hyperglycemia (18moA1C $P < 0.03$, GluMean $P < 0.02$) and glucose variability (average MAGE $P < 0.001$, average SD $P < 0.002$). The correlations with glucose variability remained significant when controlled for GluMean (average MAGE $P < 0.02$, average SD $P < 0.05$). Significant regional between-group differences (control group > group with diabetes) for cortical volume growth were found for multiple regions in the left and right hemispheres (Fig. 2 and Table 3). Significant regional between-group differences (control group > group with diabetes) for SA growth were found in right temporal pole, pars triangularis, pars opercularis, and lateral orbitofrontal regions (all $P < 0.005$). Significant regional between-group differences for thickness change (with more cortical thinning for type 1 diabetes) were found for left supramarginal gyrus ($P < 0.02$) and right supramarginal, inferior parietal, inferior frontal, lateral orbitofrontal, and precentral gyri (all $P < 0.0001$). Regional curvature was significantly negatively correlated with 18moA1C for bilateral clusters (peak locations at −18, −88, 19 and 42, −80, 6 in Montreal Neurological Institute [MNI] space) in the occipital lobes.

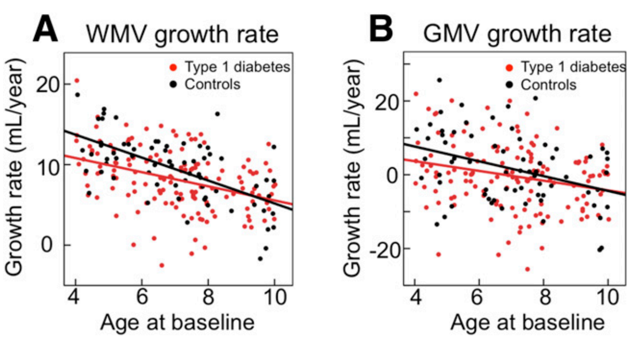

Figure 1—Growth rates of total WMVs and GMVs are significantly reduced for type 1 diabetes relative to control subjects; total WMV $P < 0.003$ (*A*) and total GMV $P < 0.05$ (*B*). Growth rate is defined as the volume at time2 minus the volume at time1 divided by the interval between scans (type 1 diabetes, red line; control subjects, black line).

Regional White Matter Growth

The children with diabetes had a significantly lower growth rate than control subjects for every regional WMV, including cerebral white matter (left $P < 0.049$, right $P < 0.004$), cerebellar white matter (left $P < 0.01$, right $P < 0.02$), brain stem ($P < 0.003$), and corpus callosum ($P < 0.001$) (Table 2). Nevertheless, for every white matter region, the children with type 1 diabetes showed significant growth from time1 to time2. FreeSurfer divides the corpus callosum into five regions, and significant growth differences were found for the posterior splenium ($P < 0.01$) and central regions ($P < 0.004$), but not for the anterior regions. At time2, the right cerebellar white matter was significantly smaller for the group with diabetes relative to control subjects (right $P < 0.01$, left

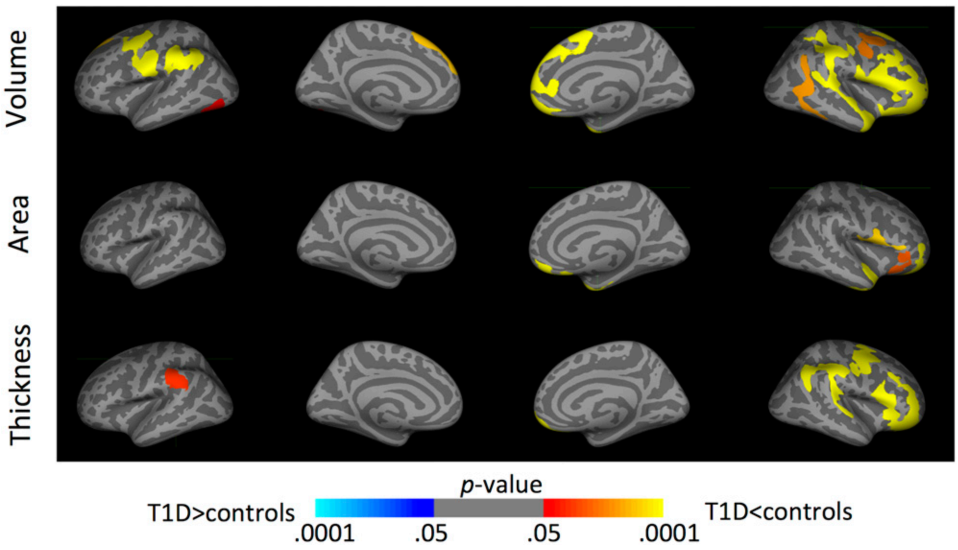

Figure 2—Cortical regions with significantly reduced growth rates for type 1 diabetes (T1D) relative to control subjects. Colors indicate surface regions where children with diabetes, relative to control subjects, have significantly less growth of cortical volume and SA and significantly more cortical thinning. Regions are displayed on an inflated cortical surface with views of left lateral, left medial, right medial, and right lateral surfaces (gyri, light gray; sulci, dark gray).

$P < 0.06$), which was the only significant cross-sectional difference between groups.

Age-Dependent Effects on Growth

The effect of type 1 diabetes on growth may vary with age, as suggested by the nonparallel regression lines in Fig. 1. A post hoc analysis, including a group × age interaction term, had a similar model fit (adjusted $R^2 = 0.23$) to the original model without it (adjusted $R^2 = 0.22$), even though the group × age interaction was not significant ($P < 0.06$). Using the interaction model, the subjects with diabetes had significantly less growth than control subjects at 1 SD

Table 3—Surface regions with significant between group differences in longitudinal gray matter change over 18 months

	Cluster size (mm^2)	Peak *t* score	Peak MNI location (x, y, z)*	*P* value**
Volume (control subjects > children with type 1 diabetes)				
L precentral, middle frontal	2,836	4.86	−52, −10, 22	0.0001
L inferior parietal, supramarginal	2,463	3.02	−49, −53, 31	0.0001
L superior frontal	1,638	2.66	−7, 57, 19	0.0004
L fusiform	896	3.49	−38, −75, −8	0.05
R frontal lobe	11,671	3.96	51, 16, 16	0.0001
R postcentral, superior temporal	5,717	5.34	47, −27, 37	0.0001
R posterior middle temporal	1,574	3.31	50, −59, 7	0.001
R caudal middle frontal	1,432	2.92	32, 7, 50	0.002
SA (control subjects > children with type 1 diabetes)				
R temporal pole	2,077	5.30	27, 7, −31	0.0001
R pars triangularis	847	3.97	42, 31, −5	0.005
R lateral orbitofrontal	1,901	3.00	20, 45, −13	0.0001
R pars opercularis	1,098	3.86	48, 7, 2	0.0003
Thickness*** (control subjects > children with type 1 diabetes)				
L supramarginal	1,185	3.40	−56, −42, 30	0.014
R supramarginal, superior temporal	3,326	4.20	47, −26, 36	0.0001
R lateral inferior frontal lobe	5,587	4.05	53, 17, 15	0.0001
R precentral	2,271	2.87	29, −2, 41	0.0001

L, left; R, right. *Peak vertex coordinates are in MNI space. **All *P* values are cluster extent corrected for family-wise error. ***Type 1 diabetes had more cortical thinning than control subjects.

below the mean age (age 5.3 years, $P < 0.001$), but not at 1 SD above the mean age (age 8.7 years, $P > 0.4$). Similarly, for total GMV, the model fit with the group × age interaction term (adjusted $R^2 = 0.04$) was similar to the model fit without it (adjusted $R^2 = 0.04$). Using the interaction model, the group with diabetes had significantly less growth than control subjects at 1 SD below the mean age (age 5.3 years, $P < 0.02$), but not at 1 SD above the mean age (age 8.7 years, $P > 0.5$).

Blood Glucose Effects on Volume

The within-subject differences of blood glucose level across time points ranged from −230 to 190 mg/dL, and were significantly negatively correlated with the change in total GMV ($P < 0.001$, slope = −93 mm^3/[mg/dL]) for volume change per change in blood glucose level (Fig. 3). Significant negative correlations were also found for the regional volumes of cortical gray matter ($P < 0.001$, slope = −76 mm^3/[mg/dL]), basal ganglia ($P = 0.001$, slope = −3.1 mm^3/[mg/dL]), hippocampus (left $P < 0.04$, slope = −0.24 mm^3/[mg/dL]; right $P < 0.006$, slope = −0.33 mm^3/[mg/dL]), and cerebellar gray matter (left $P < 0.001$, slope = −6.7 mm^3/[mg/dL]; right $P < 0.001$, slope = −6.9 mm^3/[mg/dL]) (Table 4). Similarly, the difference in blood glucose levels across time points was significantly negatively correlated with the change in WMV of the corpus callosum ($P < 0.006$, slope = −0.18 mm^3/[mg/dL]), brain stem ($P < 0.001$, slope = −1.2 mm^3/[mg/dL]), and left cerebral white matter ($P < 0.02$, slope = −6.9 mm^3/[mg/dL]). The corresponding correlation with total WMV was not statistically significant ($P < 0.06$, slope = −13 mm^3/[mg/dL]). On the cortical surface, the difference in blood glucose levels was negatively correlated with change in total SA ($P < 0.003$, slope = −6.1 mm^2/[mg/dL]) and mean CT ($P < 0.001$, slope = −0.0002 mm/[mg/dL]). The change in blood glucose level was associated with widespread surface effects for cortical volume and thickness (Fig. 4). Conversely, the difference in blood glucose levels across time points was significantly positively correlated with the change in ventricular volume ($P < 0.001$, slope = 6.1 mm^3/[mg/dL]).

Table 4—Correlation of change in brain volume, SA, and CT with difference in instantaneous blood glucose level

Region	*P* value	Regression coefficient*
Total gray matter	<0.001	−93 (18)
Cortical gray matter	<0.001	−76 (16)
Basal ganglia	0.001	−3.1 (0.90)
Left hippocampus	0.04	−0.24 (0.12)
Right hippocampus	0.006	−0.33 (0.12)
Left cerebellar gray	<0.001	−6.7 (1.4)
Right cerebellar gray	<0.001	−6.9 (1.2)
Total white matter	0.06	−13 (7.0)
Left cerebral white matter	<0.02	−6.9 (2.7)
Corpus callosum	0.006	−0.18 (0.06)
Brain stem	0.001	−1.2 (0.34)
Total SA	0.003	−6.1 (1.7) mm^2/(mg/dL)
Mean CT	<0.001	−0.00021 (0.00006) mm/(mg/dL)
Ventricular volume	<0.001	6.1 (1.3)

*Data are reported as the mean (SE). All units are mm^3/(mg/dL), unless otherwise noted.

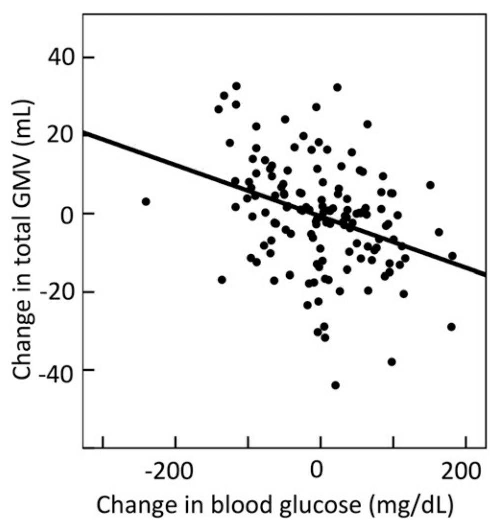

Figure 3—Change in total GMV is negatively correlated with the change in instantaneous blood glucose level across scan times for the children with type 1 diabetes (slope = −9.3 mL/[100 mg/dL], $P < 0.001$).

DISCUSSION

This longitudinal study of very young children showed that type 1 diabetes is associated with a significantly reduced growth rate in every white matter region of the brain, suggesting a widespread impact of diabetes on myelination during this period of rapid brain development (43). These children were often hyperglycemic (25), since the diabetes treatment guidelines for young children typically recommend higher glycemic targets in young children, due to their unpredictable eating and exercise patterns, underlying sensitivity to insulin, and inability to reliably communicate signs or symptoms of hypoglycemia. However, animal studies have shown that continual exposure to hyperglycemia leads to reduced myelin content and disarrangement of myelin sheaths (29,30), including in cerebellum (31). In combination, these data suggest that chronic hyperglycemia may be detrimental to the developing brain. Our results for WMV growth confirm and extend the previous results obtained using VBM analyses of the same population (25). We also emphasize that WMV is increasing for the young children with type 1 diabetes, albeit at a slower rate than that for control subjects, in contrast to adults (mean age 44 years), in whom actual losses of WMV were found for subjects with long-standing diabetes (5).

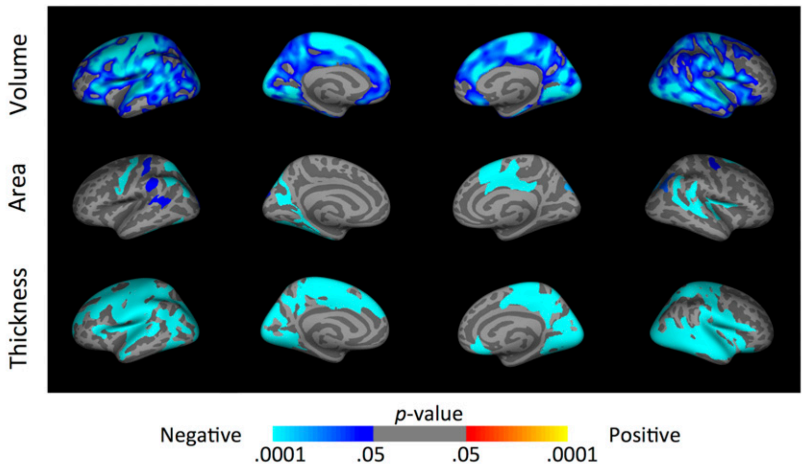

Figure 4—Cortical regions within the type 1 diabetes group with significant negative correlations to blood glucose level at the time of the scan. Colors indicate surface regions with significant negative correlations of cortical volume, SA, and CT with the change in blood glucose level. Regions are displayed on an inflated cortical surface with views of left lateral, left medial, right medial, and right lateral surfaces (gyri, light gray; sulci, dark gray).

In contrast with the current study, a similar large longitudinal study (11) of young adolescents (75 with type 1 diabetes, 25 control subjects, mean age 12.5 years) did not find significant between-group differences in total white matter growth. The difference in results between these studies cannot be explained by better diabetes control in the adolescent cohort: the mean HbA_{1c} level of 8.6% (70 mmol/mol) in the adolescent study was higher than the mean level of 7.9% (63 mmol/mol) in the current study, and the rate of severe hypoglycemic events in the adolescent study was also higher than the rate in the current study (0.6 mean events in 24 months vs. 0.05 mean events in 18 months), suggesting that better control of hyperglycemia and hypoglycemia were not responsible for the smaller structural effects observed in the adolescent age group. However, in the current study, we observed a statistical trend for a group $\times$ age interaction on growth rate such that age modified the effect of type 1 diabetes on brain growth. Specifically, for the older participants in the current study, who were similar in age to some of the young adolescents (11), there were no significant between-group differences in white matter growth rates, indicating consistent results across studies. Conversely, the significant growth differences between groups at younger ages (<7 years of age) in our study suggest that early-onset type 1 diabetes, compared with late-onset diabetes, exposes the brain to hyperglycemia during a critical early window of brain development. This age-dependent effect on structural growth may be related to the cognitive processing speed impairments specifically associated with early-onset diabetes (2,18,44). For example, animal models have shown that rats that were made hyperglycemic at 6 weeks of age, but not at 26 weeks of age, had fewer large myelinated fibers and reduced myelin width, as well as persistent reduced nerve conduction velocity relative to controls (29).

The growth of total GMV was significantly less for the group with diabetes relative to control subjects. The significant differences were found only for gray matter on the cortical surface, similar to the previous results obtained using VBM for the same young population (25). In previous studies, hyperglycemia has been associated with reduced regional gray matter growth in adolescents (10,11) and regional loss of gray matter in adults (6,7,9,14), whereas we found a widespread significant difference in the growth of total GMV in young children. Similar to the white matter analysis, there were no significant between-group differences in gray matter growth rates for the older participants in the current study. Thus, there appears to be an age-related transition from the significant between-group differences in total gray matter growth that is seen in young children, but not in young adolescents (11). However, a limitation of this result is that the group $\times$ age interaction was not statistically significant, perhaps because the experiment was not specifically powered to detect differences in age groups.

On the cortical surface, children with type 1 diabetes had significantly reduced growth of total SA relative to control subjects. The cortical surface in FreeSurfer is the gray matter-white matter boundary at the outer surface of the cerebral white matter; thus, the reduced growth of SA is consistent with the significantly reduced growth of the cerebral white matter that it encloses. Within the group with diabetes, increased glycemic exposure and higher glycemic variation over 18 months were significantly negatively correlated with the change in mean curvature of the surface, which is opposite to the longitudinal increase in curvature that was observed in the typically developing control group (Table 2). Changes in curvature have been previously reported to be an early marker of developmental changes (45). In the current study with young children, the curvature result is consistent with the reduced growth of total SA found for the population with diabetes, such that reduced area growth for a given volume of white matter could result in less curvature (or less "wrinkling") of the surface.

There were significant correlations of gray matter, white matter, and ventricular volumes with the change in blood glucose level across time points. Note that these effects were not due to longitudinal growth, but rather appeared to represent state effects where the instantaneous brain volume was associated with the blood glucose level at the time of the scan. To our knowledge, these results are the first report of a significant correlation between instantaneous blood glucose levels and GMVs and WMVs in a young population with type 1 diabetes. In healthy adults, changes in ventricular volume have been observed within 30 min of ingestion of a glucose drink (46), and enlarged ventricles have been found for adults with recent-onset type 2 diabetes relative to an age-matched population (47). In the current study, children with diabetes had large diurnal glucose fluctuations (average MAGE 159 mg/dL). The estimated correlations between brain volumes and blood glucose level suggest that a 159 mg/dL increase in blood glucose level would decrease total brain tissue volume (gray plus white matter) by 16.9×10^3 mm^3, or 1.4% of the average total brain volume of $1{,}180 \times 10^3$ mm^3 seen in our study. Similar percentage changes in total GMV and WMV have been associated with dehydration and rehydration in healthy adults (32,33). Importantly for the current study, the effect of current blood glucose level on brain volume did not confound the longitudinal between-group growth effects because the mean HbA_{1c} levels, MAGE levels, and blood glucose levels of the group with diabetes were the same across time points (Table 1). In addition, the most significant volume fluctuation effects were seen in gray matter, while the most significant between-group growth effects were seen in white matter.

The correlations between blood glucose and brain volume raise the question of whether the brain of an individual with diabetes undergoes more osmomechanical stress from continued compression and expansion than a typically developing brain. In particular, the glucose variation in children with diabetes (mean MAGE 159 mg/dL) is more than four times larger than that in healthy adults (mean MAGE 35 mg/dL) (48). While typical levels of osmomechanical stress generally preserve strong neuronal connections (49), it may be possible that the larger levels of volume fluctuations seen in children with diabetes may disrupt the connections of weaker new synapses, particularly in developing gray matter. However, an important limitation of our longitudinal volumetric analysis is that it does not differentiate among diurnal glycemic or osmotic fluctuations, recent hydration effects, or long-term glycemic history. Future controlled measurements are recommended to better understand these osmotic effects.

In summary, we found that children with type 1 diabetes, relative to control subjects, had significantly reduced growth of total cortical GMV and SA, as well as of all white matter regions throughout the cerebral cortex and cerebellum. The growth differences were most pronounced at the younger end of the age range, and there was a consistent transition to the more limited regional growth effects from type 1 diabetes that were previously reported for adolescents. In addition, our data suggest that large fluctuations in glucose levels (perhaps mediated by osmotic effects) may be associated with corresponding fluctuations in GMVs and WMVs, introducing another potential stress on brain development. Of note, the children with diabetes in this study were relatively well controlled, with an average HbA_{1c} level of 7.9% (63 mmol/mol) at each time point. This level compares favorably to recent large-scale registry studies (20) in the U.S., in which average HbA_{1c} levels were almost 0.4% (4.4 mmol/mol) higher. It is conceivable that even larger brain differences may have been found had we studied children with higher GluMean and HbA_{1c} levels. It is becoming increasingly clear that tighter management of both GluMean levels and glycemic fluctuations may be beneficial for brain growth. Further studies of these effects may help to elucidate their impact on brain development and the mechanisms associated with these effects.

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Author Contributions. P.K.M. and S.A.W. researched and analyzed the data and wrote the manuscript. N.M., B.B., N.H.W., E.T., T.H., A.C., T.A., L.F., D.M.W., M.J.T., and W.T. researched the data, contributed to discussion, and reviewed and edited the manuscript. D.P., M.R., and M.M. analyzed the data, contributed to discussion, and reviewed and edited the manuscript. A.L.R. researched and analyzed the data, contributed to discussion, and reviewed and edited the manuscript. P.K.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix

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